MECHANISMS OF VESTIBULAR ION TRANSPORT

(NAG 2-404)

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Summary of Project

The overall aim of this project was to provide evidence regarding the possible function and regulation of Na^+,K^+-ATPase in the vestibular periphery. It was initially considered that losses of body fluid and electrolytes, which occur upon exposure to microgravity, may alter vestibular Na^+,K^+-ATPase activity by mobilization of endocrine agents regulating fluid and electrolyte balance. One specific question was whether vestibular potassium-transporting dark cell epithelia were subject to regulation by *mineralocorticoids* such as aldosterone, i.e., whether aldosterone could regulate the population density of the Na^+,K^+-ATPase enzyme sites and whether the presence of mineralocorticoid receptors could be demonstrated in the dark cell epithelium. Another question was whether *glucocorticoid* receptors were present in vestibular tissues. A third question concerned the vestibular Na^+,K^+-ATPase -- would it be elevated in vestibular hair cells and nerve fibers, suggesting a role for the enzyme in the specialized function of cellular transduction? A final question was whether presumptive cholinergic efferent neurons, which end on the vestibular sensory and neural elements, utilize muscarinic acetylcholine receptors, possibly in the modulation of vestibular afferent discharge.

These questions were all addressed in the present grant, and a number of interesting findings emerged. The effect of alteration of serum aldosterone levels on Na^+,K^+-ATPase in ion-transporting regions of the guinea pig inner ear was investigated by utilizing a high Na^+-low K^+ diet to first significantly lower aldosterone in the animals. An injection of aldosterone 21 hours prior to sacrifice resulted in significant elevation of serum aldosterone over that obtained with a high Na^+-low K^+ diet. Binding of ^{3}H-ouabain, a specific inhibitor of Na^+,K^+-ATPase, was significantly increased in microdissected ampullae of the semicircular canals for aldosterone-injected vs. vehicle-injected animals. Serum $[Na^+]$ and $[Cl^-]$ were elevated in animals on the high Na^+-low K^+ diet and unaltered by administration of exogenous aldosterone, indicating that the enhancement of ouabain binding in inner ear tissues observed with aldosterone-injected animals did not reflect an alteration of serum electrolytes *per se*. The results of these experiments are consistent with the hypothesis that the population of Na^+,K^+-ATPase sites increases in response to aldosterone in ion-transporting epithelia of the semicircular canal.

In more recent studies, presumptive mineralocorticoid (Type I) receptors were investigated by measuring binding of ^{3}H-aldosterone in the semicircular canal of male Hartley guinea pigs. These experiments demonstrated the presence of significant aldosterone binding, suggesting the existence of mineralocorticoid receptor sites. Thus, the vestibular tissues appear to be a target site for mineralocorticoid action. Presumptive glucocorticoid (Type II) receptors were also investigated by employing the synthetic glucocorticoid ^{3}H-RU 28362, which has negligible binding to mineralocorticoid receptors. Substantial specific binding of ^{3}H-RU 28362 to ampullar tissues was observed, providing evidence for glucocorticoid receptors in the vestibular periphery. Such receptors may serve as sites of glucocorticoid action.

It was further demonstrated that Na^+,K^+-ATPase activity is elevated in dark cells of the utricle and ampullae (the dark cells have characteristic membrane infoldings on their basolateral surfaces, suggestive of fluid and ion transport). In these studies, vestibular organs were isolated from gerbil inner ear, and processed by the cytochemical method of Ernst (*J. Histochem. Cytochem.* 20: 13-22, 1972) for electron microscopic demonstration of Na^+,K^+-ATPase. No enzyme activity detectable with the cytochemical procedure could be found in epithelium of the saccular wall, although copious reaction product was associated with dark cells of the utricle and ampullae. The saccular endolymphatic compartment is isolated from the utricle and ampullae, but communicates more freely with the cochlear duct. It therefore seems likely that the cation content of saccular endolymph, in contrast to the situation for the utricle and ampullae, is maintained primarily by the marginal cells of the cochlear lateral wall.

Finally, vestibular organs were isolated from gerbil inner ears by microdissection, labeled with [^{3}H]quinuclidinyl benzilate at 1 nanomolar concentration, and processed for light-microscopic autoradiography. Autoradiographic observations indicated that the highest grain densities were associated with the ampullae. Both in the ampullae and macular organs, autoradiographic label was concentrated within the sensory epithelium at a level corresponding to the synaptic poles of the hair cells. Additional label was found over nerve fibers passing through the connective tissue beneath the sensory epithelia. This last study suggests the presence of cholinergic receptor sites, presumably muscarinic, in vestibular sensory organs.

Publications Resulting from NASA Grant NAG 2-404

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